Claims

1. A method of preparing controlled release microspheres comprising the steps of:

(a) forming an emulsion comprising an aqueous dispersed phase, said dispersed phase comprising:

- a polymer capable of forming a hydrogel,
- a bioactive protein, and
- water,

and subsequently

(b) crosslinking the polymer physically or chemically to form a hydrogel;

wherein the aqueous dispersed phase is substantially free from insoluble aggregates of said bioactive protein.

2. A method of preparing controlled release microspheres comprising the steps of:

(a) providing a first aqueous phase comprising a polymer capable of forming a hydrogel, a bioactive protein, and water;

(b) providing a second aqueous phase comprising a compound which is capable of phase separation when combined with the polymer capable of forming a hydrogel, and water;

(c) forming an emulsion by dispersing the first aqueous phase in the second aqueous phase; and subsequently

(d) crosslinking the polymer capable of forming a hydrogel physically or chemically to form a hydrogel;

wherein the water content of the second aqueous phase is at least at approximate equilibrium with the water content of the first aqueous phase.

3. A method of preparing controlled release microspheres comprising the steps of:

(a) providing an aqueous phase having a temperature T_1, said phase comprising an amount of:

- a bioactive protein;
- a polymer capable of forming a hydrogel;
- a compound which is capable of phase separation when combined with the polymer capable of forming a hydrogel; and
- water;

wherein the amounts are selected to yield an aqueous single phase system at T_1, but a two-phase system at T_2, and wherein T_2 is lower than T_1;

(b) cooling the aqueous single phase system provided in step (a) from T_1 to T_2, thereby inducing phase separation and the formation of an emulsion; and subsequently

(c) crosslinking the polymer capable of forming a hydrogel physically or chemically to form a hydrogel.

4. The method of any one of the preceding claims, wherein the polymer capable of forming a hydrogel is a prepolymer.

5. The method of any one of the preceding claims, wherein the polymer capable of forming a hydrogel is capable of being physically crosslinked by crystallisation or stereocomplex formation.

6. The method of any one of the preceding claims, wherein the polymer capable of forming a hydrogel is a polysaccharide or modified polysaccharide, and preferably a modified dextran.

7. The method of claim 6, wherein the polymer capable of forming a hydrogel is selected from dextran hydroxyethylmethacrylate (dexHEMA), dextran hydroxypropylmethacrylate (dexHPMA), dextran

hydroxypropylmethacrylamide (dexHPMAm), and dextran hydroxyethylmethacrylamide (dexHEMAm).

8. The method of any of the preceding claims, wherein the bioactive protein has a solubility of less than about 10 wt.-% in water or physiological buffer solution at room temperature.

9. The method of any of the preceding claims, wherein the bioactive protein is selected from the group consisting of insulin, epoetin-alfa, epoetin-beta, calcitonin, heparin, IFN(interferon)-alfa-2a, IFN-alfa-2b, PEG-IFN-alfa, IFN-alfacon-1, IFN-beta, IFN-beta-1a, IFN-beta-1a, IFN-beta-1b, IFN-gamma-1b, somatropin, follitropin, menotropin, leuprolide, goserelin, buserelin, triptorelin, filgrastim (G-CSF), lenograstim (G-CSF), sargrarmostim (GM-CSF), PEG-G-CSF, interleukins, blood clotting factors such as factor VIII and factor IX, nadroparin, dalteparin, tinzaparin, certoparin, reviparin, tirofiban, octreotide, antigens, and monoclonal antibodies.

10. The method of any of the preceding claims, wherein the aqueous phase which comprises the bioactive protein also comprises an excipient which is capable of reducing the aggregation of the bioactive protein.

11. The method of claim 10, wherein the excipient is selected from the group consisting of surfactants, sugars, sugar alcohols, chaotropic agents, antioxidants, amino acids, and inorganic salts.

12. The method of any of the preceding claims, wherein the step of crosslinking the polymer capable of forming a hydrogel is conducted within about 15 minutes after the formation of the emulsion.

13. The method of any of the preceding claims, wherein the step of forming an emulsion is conducted as a continuous process.

14. The method of any of the preceding claims, further comprising any of the following steps:

(a) collecting the microspheres;

(b) purifying the microspheres; and/or

(c) drying the microspheres.

15. The method of claim 2 or 3, wherein the compound which is capable of phase separation when combined with the polymer capable of forming a hydrogel is polyethylene glycol.

16. The method of claim 1, wherein the aqueous dispersed phase comprises about 5 to 60 wt.-% polymer or prepolymer, and about 1 to 30 wt.-% bioactive protein.

17. The method of claim 1, wherein the emulsion comprises an aqueous continuous phase, said aqueous continuous phase comprising a compound which is capable of phase separation when combined with the polymer capable of forming a hydrogel, and water, which compound is preferably polyethylene glycol.

18. The method of claim 1, wherein the step of forming the emulsion comprises the substeps of:

(a) providing the bioactive protein in a solid, soluble form; and

(b) combining the bioactive protein with an amount of water.

19. The method of claim 18, wherein the amount of water is selected to yield a concentration of the bioactive protein which is about equivalent to, or higher than the concentration of the bioactive protein in the dispersed phase of the emulsion.

20. The method of claim 18 or 19, wherein the amount of water is provided in form of an aqueous solution or dispersion of a compound which is

capable of aqueous phase separation when combined with the polymer capable of forming a hydrogel.

21. The method of any of claims 18 to 20, wherein the bioactive protein is provided in lyophilised form.

22. The method of claim 18 to 21, wherein the bioactive protein is provided as a lyophilised mixture comprising the polymer capable of forming a hydrogel.

23. The method of any of claims 18 to 22, wherein the bioactive protein is provided as a soluble precipitate.

24. The microspheres obtainable by the method of any of the preceding claims.

25. .Controlled release microspheres comprising a biodegradable, physically or chemically crosslinked polymer and a bioactive protein, being substantially free from insoluble aggregates of said bioactive protein.

26. The microspheres of claim 24 or 25, wherein the crosslinked polymer is derived from a polysaccharide, and preferably from a dextran or dextran derivative.

27. The microspheres of claims 24 to 26, comprising about 0.1 to 60 wt.-% bioactive protein.

28. The microspheres of any of claims 24 to 27, wherein a fraction of at least about 95 wt.-% of the bioactive protein is dissolvable and releasable from the microspheres under physiological conditions.

29. The use of the microspheres of any of claims 24 to 28 as carriers for therapeutic or diagnostic bioactive proteins.

30. Pharmaceutical composition for the controlled release of a bioactive protein, comprising the microspheres of any of claims 24 to 28.

31. The composition of claim 30 being provided in dry and sterile form.

32. The composition of claim 31, being formulated and processed to be suitable for parenteral injection.

33. The composition of claim 32, wherein the microspheres have an average diameter of about 1 to about 100 μm, as determined by laser diffraction.

34. The composition of claim 30, being formulated and processed to be suitable for inhalation.

35. The composition of claim 35, wherein the microspheres have an average diameter of about 1 to about 20 μm, and more preferably of about 2 to about 10 μm, as determined by laser diffraction.

36. The composition of claim 34 or 35, wherein at least about 80 wt.-% of the microspheres have a diameter between about 2 and 10 μm, as determined by laser diffraction.